ON GASTRULATION AND THE ORIGIN OF THE PRIMITIVE STREAK IN THE PIGEON'S EGG. — PRELIMINARY NOTICE.

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The results of the experimental studies of Assheton ('96), Miss Peebles ('98), and Kopsch ('02) on the primitive streak of the chick demonstrate beyond any reasonable doubt that the material of that structure enters into the formation of the embryo — a view long held by many embryologists. In the light of these experiments the opposite view of Balfour and his followers is no longer tenable. The results obtained by these three workers have, in the main, solved the problem of the fate of the primitive streak, but they have not answered the question of its origin. The present work was undertaken with the hope of throwing light upon the latter question. It was soon found that its solution depended upon a morphological and experimental study of stages occurring before the time of laying. The morphological results mainly will be considered in this paper.

Material and Methods.

It is doubtful if a more desirable material could be found for the purposes of this investigation than that furnished by the pigeon's egg. The regularity of the laying habits of the common pigeon makes it possible to secure eggs at approximately any stage of development. Breeders have long known that this bird ordinarily lays two eggs at a sitting, the first usually between four and six P. M., and the second between one and two P. M., on the second day following. According to Harper ('04) this latter egg is fertilized at about eight P. M., just before it enters the oviduct, and hence it is forty-one hours in traveling down this passage. Thus, it will be seen that the investigator can secure this second egg at approximately any stage of its early development, for he needs but kill the bird at the proper hour and remove the egg from the oviduct in order to obtain a desired stage. Eggs removed in this manner, even as early as twenty hours before lay-

ing, can be used for experimentation; for by this time the shell is firm enough to permit handling without injury to the blastoderm.

In dealing with this material it was necessary to use special technique both for fixing and orientation. The picro-sulphuric-acetic mixtures have been found to be vastly superior to all other reagents. Of these mixtures the most successful is 92 parts of Kleinenberg's strong picro-sulphuric plus 8 parts of glacial acetic. The whole yolk is immersed in this fluid for one hour and is then treated with 70 per cent. alcohol for several hours, after which it is placed in 80 per cent. At this point it is found advisable to cut out a properly oriented wedge-shaped block of yolk containing the blastoderm, with vitelline membrane still attached. After completely washing out the picric acid, this block is carried through the higher alcohols, cleared in cedar oil, and embedded and sectioned in the usual way.

For stages prior to the appearance of the primitive streak, such a treatment necessitates a careful orientation of the blastoderm before using the fixing fluid. Already a method for orienting the chick blastoderm has been worked out. Thus a number of investigators have shown that if a hen's egg be held in front of the observer so that the blunt end is to his left and the pointed end to his right, the posterior margin of the blastoderm will be towards and the anterior away from him, and hence, when the embryo appears, its head will be directed away from the observer, with its long axis meeting the chalazal axis at right angles. If a pigeon's egg be held in a similar position a different condition is found. The posterior margin of the blastoderm, instead of being directly in front of the observer, is forty-five degrees to his left, and when the embryo arises, its long axis meets the short axis of the egg at an angle of forty-five degrees (see Fig. 1).

For some purposes iron hæmatoxylin has been of great value as a stain, but for general use a modification of Delafield's hæmatoxylin is unsurpassed, especially for demonstrating the presence of cell walls.

Gastrulation.

I stated above that it was necessary to investigate the period of development that occurs before laying. A study of these early stages naturally involves the question of the origin of the two

primary germ layers. Concerning the manner in which these two layers arise there has been a wide difference of opinion among embryologists, although a great deal of attention has been paid to this question. The unsatisfactory solution of this problem is due to the fact that most of the conclusions are based on incom-

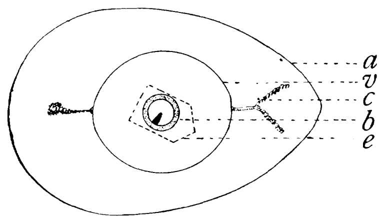

FIG. 1. Scheme for orienting the blastoderm of the pigeon's egg in cutting sections. *a*, shell; *b*, blastoderm at the first appearance of the primitive streak; *c*, chalaza; *v*, vitelline membrane; *e*, wedge-shaped block of yolk containing the blastoderm which is cut out and embedded for sections.

plete evidence. So far as I am aware, not a single observer has had a complete series of normal stages of any one type from which to draw his conclusions. The divergent views as to the origin of the entoderm, however, can be grouped into three classes. (1) A number of the older workers have maintained that it arises by a process of delamination, that is, the upper cells of the segmented disc arrange themselves into a continuous layer, constituting the primary ectoderm, while the deeper cells of the disc form the primary entoderm. This view is not in accord with what is known to occur in many other forms. (2) Others have maintained that the entoderm arises by an ingrowth of cells into the segmentation cavity from a part or all of the inner edge of the germ-wall. The most recent contribution supporting this view is by Nowack ('02) who states that the bulk of the entoderm is formed out of a mass of cells, which grows forward from the posterior part of the germ-wall. In speaking of this forward growth he says: "Es gehen nämlich von der Gegend des hinteren Keimwalles, als unmittelbare Fortsetzung desselben, kurze Zellstränge aus, die mitten durch die Keimhöhle nach vorn ziehen, miteinander in Verbindung treten und eine dünne Platte von verschiedener Dicke und vielen grösseren und kleineren Löchern bilden. Diese Platte endet vorn und an den Seiten mit freiem,

wenn auch unregelmässigem Rande, zeigt also ein zungenförmiges Aussehen. Man wird wohl nicht fehlgehen, dieses Gebilde als den Anfang der unteren Keimschicht, d. h. des Entoderms zu bezeichnen. Es ist dies allerdings nicht das einzige zellige Material, was innerhalb der Keimhöhle zu finden ist, aber doch die bei weitem grösste Menge."[1] (3) The third class includes those who believe that the entoderm arises by a process of gastrulation, that is, the upper layer turns under to give rise to the lower layer. This view has been supported by Haeckel, Goette, Rauber, and others. The work of Duval ('84) also has been quoted in support of gastrulation. This author describes the blastoderm at the end of segmentation as a biconvex lens (lentille biconvexe), in which two layers can be recognized ; an upper epithelium-like layer separated by a narrow fissure from a thick lower layer. The deepest cells of the latter are open below to the white yolk of the Nucleus of Pander. In a later stage a thickening occurs on the margin where the upper layer is united with the lower. Duvals calls this thickened rim the *bourrelet blastodermique*. It corresponds to the *Randwulst* of the German authors. At the posterior margin where the rim is thickest, a crescent-shaped groove appears, which passes forward beneath the blastoderm as a fissure separating the lower cells of the blastoderm from the underlying yolk. Duval now regards the blastoderm as in the gastrula stage and hence the fissure between the yolk and the thick lower layer is the archenteron. It is clear that, in the main, Duval's theory is one of delamination. So far as the pigeon's egg is concerned the segmentation cavity is *not found* just below the superficial layer of cells at the end of segmentation, but is situated beneath the central portion of the blastoderm — between the deepest cells and the yolk.

In order to work out the history of a continuous developmental process, such as gastrulation, it is necessary to have a complete series of normal stages taken from one type. Such a series is easily obtainable from the pigeon, and the following account of gastrulation is based upon a study of several series of this birds's egg.

In seeking for a stage at which to begin the account of gastru-

[1] *Loc. cit.*, p. 27.

lation, I found the close of segmentation to be the most advantageous time, for it is shortly after this period that the first direct steps leading up to invagination occur. At the close of segmentation the disc is three or four cells deep, except at the extreme margin where it gradually diminishes to a thickness of one or two cells. Beneath and external to the marginal cells of the disc, yolk or "periblastic" nuclei are present. According to Miss Blount ('07) these nuclei segregate about themselves the neighboring protoplasm, and later, cell walls appearing are added to the disc, thus contributing to its extension. Waldeyer ('69), Hertwig ('99), and others have advanced similar views for the chick and selachian, designating it supplementary cleavage. Where these cells are being added to the disc a more or less syncytial condition exists around the entire margin. This region constitutes the germ-wall.

Shortly after the period described above there occurs the first direct step in the process of gastrulation. This is in the nature of a thinning of the posterior part of the segmented disc. This process begins, not at the extreme margin, but usually slightly posterior to the center, and then spreads in all directions, but with more rapidity towards the posterior margin.[1] The first stage of this process is shown in Fig. 2. Slightly posterior to the center and almost directly above the segmentation cavity, the disc is but two cells deep, while in the region of the germ-wall it is four deep. The characteristic features given above for the germ-wall and the extension of the disc can also be made out from this figure. At this time the segmentation cavity is still very shallow, but upon further progress of the thinning out it becomes much more extensive, and may then be called the subgerminal cavity.

As the thinning out progresses the germ-wall becomes interrupted in the posterior region, as is shown in Fig. 4. The blastoderm from which this drawing was made is much more advanced than that in Fig. 2, being about eleven hours older. The changes occurring between these two stages, however, are gradual and may be followed with comparative ease. In the first place there is a very rapid division of cells, as is evidenced by

[1] *In Torpedo ocellata* Zeigler ('02) describes the thinning-out of the blastoderm as beginning at the posterior and progressing anteriorly.

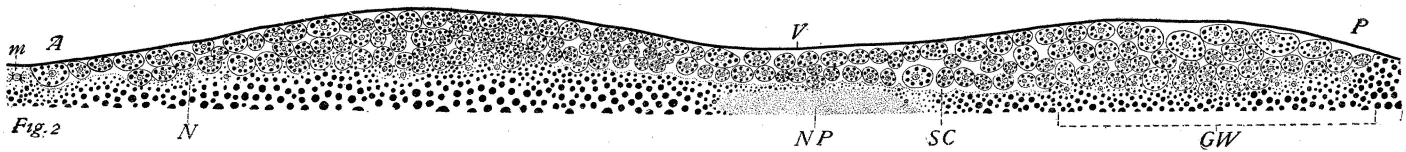

FIG. 2. A median longitudinal section of a blastoderm taken twenty-one hours after fertilization, or twenty hours before laying. *m*, a periblastic nucleus in the process of division. $\times 77.3$.

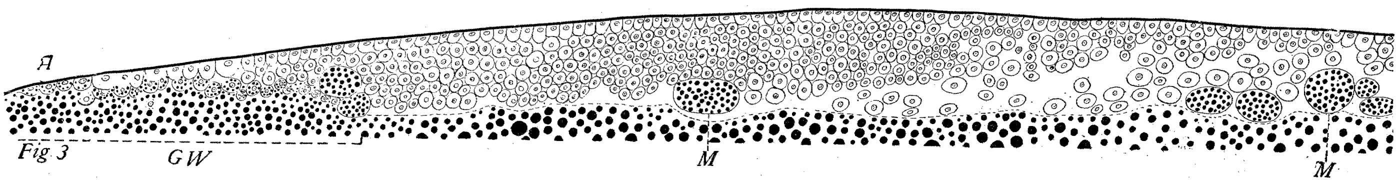

FIG. 3. Anterior half of a median longitudinal section of a blastoderm taken thirty-one hours after fertilization, or ten hours before laying. $\times 129.5$.

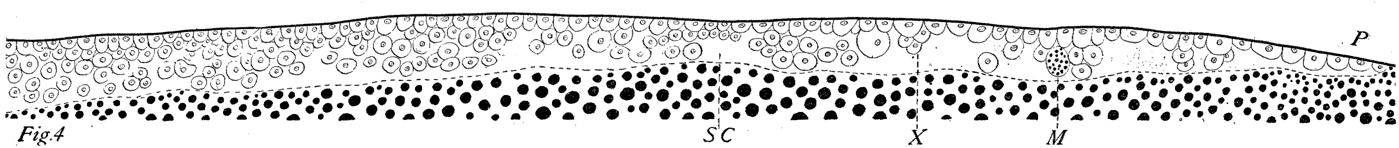

FIG. 4. Posterior half of the same section as represented in FIG. 3. This figure shows the condition of the posterior part of the blastoderm just before invagination begins. *X*, cells crowding into the ectoderm. $\times 129.5$.

their number and size in the anterior half of the blastoderm (Fig. 3), where they are about seven layers deep. However, as one passes from the anterior to the posterior margin there is a gradual change in depth from seven cells to one. There are also found in the anterior region large yolk masses (Fig. 3, *M*), which arise from the floor of the segmentation cavity. At this stage they are not limited to this region, but occasionally are found in the posterior half (Fig. 4, *M*), where the disappearance of the germ-wall is one of the most characteristic features. This interruption of the germ-wall goes hand and hand with the thinning out, which is rapidly establishing a one-layered condition of the blastoderm. In other words the phenomenon of thinning-out is nothing more nor less than the crowding of the cells of the segmented disc into a single layer. It is evident that this must result in a rapid centrifugal expansion of the blastoderm. That this is actually the case is shown by measurements. Thus at twenty hours after fertilization the average diameter of the blastoderm is 1.915 mm., while at thirty hours it is 2.573 mm. In fact there is no other period in the early history of the blastoderm in which there is such a rapid increase in the surface area, as occurs during the time when the thinning out is at its maximum. One would not be justified, however, in saying that this entire expansion is brought about by the thinning out, for according to Miss Blount's interpretation the germ-wall is also contributing materially to this increase.

In the posterior third of this blastoderm the single layer is almost complete; still, at places, some of the few cells yet remaining in the segmentation cavity can be seen apparently in the act of crowding up into the single layer (Fig. 4, *X*). Whether or not, in all cases these remaining cells eventually succeed in getting into the upper layer, approximately above where they are situated is not clear. They do in the majority of blastoderms, but I have some few series in which they seem to migrate anteriorly and to the sides, where the last stages of thinning out occur. In either case they take no part in the formation of the gut-entoderm.

In Fig. 5 is shown a reconstruction from sections of the blastoderm represented in Figs. 3 and 4. The germ-wall does not

completely encircle the rim of the blastoderm, but it is interrupted for a distance of about 70 degrees at the posterior margin. The form of the germ-wall is that of a crescent, with its horns stopping slightly short of the area over which a single layer has been developed.

During the two or three hours immediately following the stage just described, the thinning out continues to extend anteriorly over the central portion of the blastoderm, and at the same time spreads laterally. By the time the posterior third of the blasto-

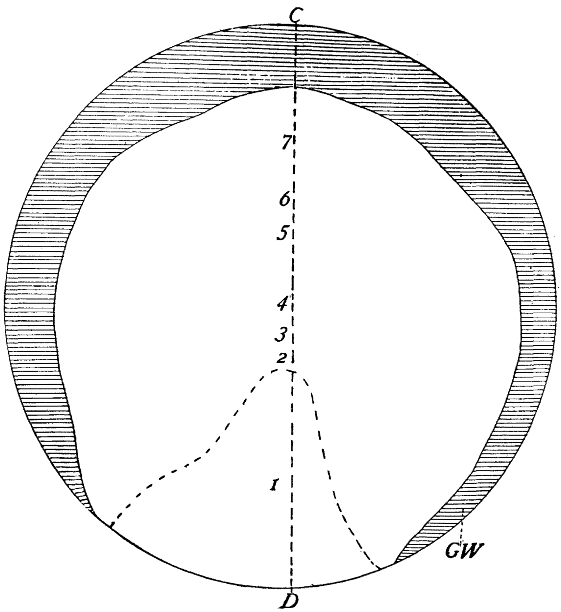

FIG. 5. A diagrammatic reconstruction from sections of the blastoderm from which Figs. 3 and 4 were drawn. *GW*, germ-wall. Numbers 1, 2, 3, etc., represent the regions of the blastoderm which are one, two, three, etc., cells deep, respectively. The broken line around 1 indicates the region where the blastoderm is approximately one cell deep. × 27.2

derm is thinned out to approximately one layer there occurs the initial step in gastrulation. Owing to the individual variation in the development of eggs, some difficulty has been experienced in securing a complete series through this brief period, so that at present I am not in a position to state positively in what this initiatory step consists. The evidence, however, inclines me to believe that it is brought about by a rolling under of the thin

free edge of the posterior margin of the blastoderm. This interpretation is in harmony with what is known to occur in the gastrulation of other forms, especially the fish. Thus Agassiz and Whitman ('84) state that in *Ctenolabrus* "there is a plain rolling under, or involution, as an initiatory step in the formation of the ring," but they believe that it is more correct to describe the process "as an ingrowth, due both to a rapid multiplication of the cells, and also to the centrifugal expansion of the ectoderm." There are certain differences between the teleost and pigeon blastoderms which, in this connection, must not be overlooked. Thus at the time of invagination the teleost blastoderm is three or four cells thick, and the epidermal layer of the ectoderm takes no part in the involution. On the other hand, the pigeon blastoderm is approximately but one cell thick at the posterior margin where invagination occurs, and hence all the cells of this margin participate in the involution. The interpretation of sections certainly supports this view, but the appearance of sections is often misleading. Two other sources of evidence are much more convincing. In the first place, careful measurements show that previous to and following gastrulation the blastoderm is nearly circular, but during the period of invagination the antero-posterior diameter is *always shorter* than the transverse diameter. This is exactly what one would expect if the posterior margin turns under instead of growing out over the yolk. In the second place an injury made on the posterior margin of the blastoderm during the early stages of invagination is found, upon further incubation, in the gut-entoderm, that is, it has been carried down under the blastoderm.

There occurs simultaneously with the turning under of the free edge a rapid thickening in the region of invagination, that is, on the posterior border where the upper layer turns under to become continuous with the invaginated portion. This thickening is not to be accounted for merely by a multiplication of cells *in situ*, but is largely brought about by a movement of material to the median axis from the lateral portions of the posterior margin of the blastoderm. This shifting of material necessarily brings about the approximation of the horns of the germ-wall, and in thus approaching each other they finally meet, and thus close the

blastopore. All this will become clear upon examining a blastoderm during the period in which gastrulation is at its height, and comparing it with a stage such as is shown in Fig. 5.

Fig. 6 is a reconstruction of a blastoderm at the height of gastrulation, which was taken five hours before laying, or thirty-six hours after fertilization. It represents a dorsal view as though the ectoderm were transparent. On the anterior and lateral margins is a clear crescent-shaped area (O), the region of over-growth.

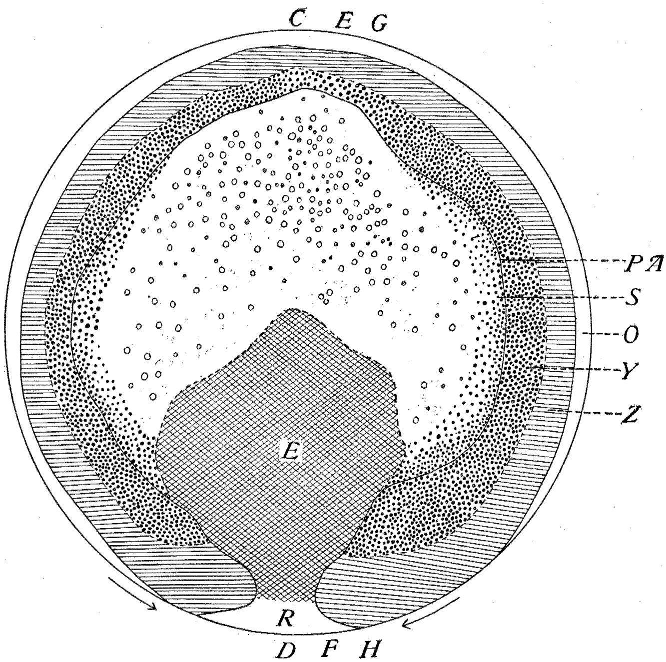

FIG. 6. A diagrammatic reconstruction of a blastoderm taken thirty-six hours after fertilization, or five hours before laying. It represents the ectoderm as transparent. *O*, region of overgrowth; *Z*, zone of junction; *Y*, yolk zone; *PA*, outer boundary of the area pellucida; *S*, beginning of yolk-sac entoderm; *E*, region covered by invaginated or gut-entoderm; *R*, dorsal lip of the blastopore. Lines drawn through *CD*, *EF*, and *GH* represent the planes of the sections illustrated in Figs. 8, 9 and 10, respectively. The arrows at posterior margin indicate the direction of movement of the halves of the margin. $\times$ 27.2.

Between this area and the subgerminal cavity (PA) is the germ-wall, in which two distinct zones can be recognized. The outer of these (Z) may be designated as the *zone of junction*,[1] and is

[1] This term was first used by Agassiz and Whitman, '84.

characterized by a fusion of layers, in which there may be a more or less syncytial condition. Above the region of the inner or *yolk zone* a distinct ectoderm is present, below which are found many large cells heavily laden with yolk. These cells, which may be more or less surrounded by yolk, in which numerous "periblastic" nuclei are present, are destined to become the yolk-sac entoderm. This zone is the region formerly occupied by the zone of junction.

Within the subgerminal cavity are important structures. Scattered over the greater part of its area, but more numerous in the anterior region, are many large yolk masses, among which are also a few of the remaining segmentation cells that have not yet succeeded in getting into the ectoderm. At the extreme sides of the cavity are a great number of cells (*S*), which in the main are the same as the latter. However, it is possible that some of these may have been given off from the inner edge of the germ-wall. *E*, a tongue-like process, is the region over which the invaginated entoderm extends. It reaches from near the posterior margin to slightly beyond the center of the blastoderm. Its anterior and antero-lateral margins end freely, but its postero-lateral margins are bounded by the horns of the germ-wall. At the extreme posterior the entoderm is in connection with the thickened rim, a region of indifferent structure. Where the entoderm arises from the rim it is necessarily thick but it gradually thins out anteriorly. Beneath this rim (*R*) is a passage, the blastopore, which is widest at the margin, gradually narrowing as it passes toward the center. This passage becomes continuous with the cavity under the entoderm — the archenteric cavity.

Sections of this blastoderm are very instructive. A portion of the anterior half of a section, four sections to the left of the median line, is shown in Fig. 7. At the extreme anterior margin is the region of overgrowth (*O*), where the blastoderm first spreads over the yolk. The width of the overgrowth never exceeds that shown in this series, for as fast as it extends out over the yolk the germ-wall keeps pace with it. In the more peripheral portion of the germ-wall separate layers cannot be distinguished. This is the zone of junction, which seldom has a greater width than is shown in this figure. But next to the

subgerminal cavity a distinct ectoderm can be recognized, and just beneath this ectoderm are cells mingled with yolk granules in which periblastic nuclei are present. As the overgrowth proceeds over the yolk, followed by the zone of junction, it is evident that that portion of the germ-wall between the zone of junction and the subgerminal cavity (*SG*), that is, the yolk zone (*Y*), will continue to increase in width. But the cavity, due to the liquefaction of yolk, is also increasing in width, but at a slower rate. In this widening of the cavity there are left around its margin cells which were previously embedded in the yolk. These cells form the beginning of the yolk-sac entoderm, and when the invaginated entoderm has spread over the subgerminal cavity its free margin becomes continuous with that of the yolk-sac entoderm. The last place for this union to occur is in the anterior region of the cavity.

Within this region of the cavity are found many large yolk masses (*M*), in some cases so numerous as to cause an elevation of the ectoderm, especially in later stages. Against the under surface of this layer are crowded a few remaining segmentation cells (*X*). These are the cells which Gasser ('82) has mistaken for wandering entoderm cells and Nowack ('02) for wandering ectoderm cells. At the extreme right of this figure the free end of the invaginated entoderm can be seen (Fig. 7, *L*).

Fig. 8 is a portion of the posterior half of a longitudinal median section (see Fig. 6). In the posterior region is the thickened rim, or dorsal lip of the blastopore (*R*).[1] From its rounded appearance one might infer that the posterior margin is still rolling under to form the entoderm, but at this stage it is more correct to regard the entoderm as arising as a forward growth from the inner edge of the thickened rim. At the place of origin (*U*) the entoderm is very thick, but gradually thins out anteriorly. The blastopore (*B*) is a shallow passage extending inward from the exterior to become continuous with the archenteric cavity (*AC*), which is that portion of the subgerminal cavity covered by the entoderm. Within the archenteric cavity are two large yolk masses (*M*) which have just risen out of the yolk. Below the

[1] The ventral lip is represented by the yolk lying immediately beneath the blastopore.

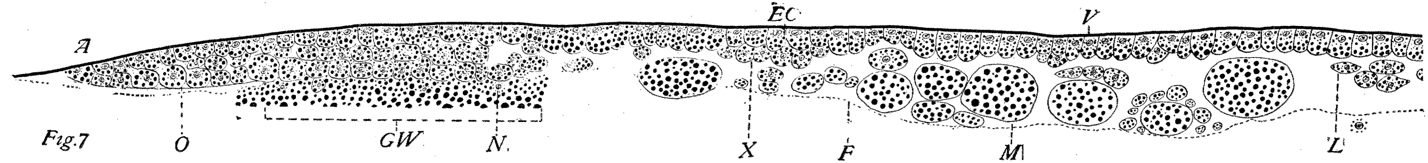

FIG. 7. Anterior portion of a section taken slightly (4 sections) to the left of the plane passing through CD of Fig. 6. X, segmentation cells crowding into the ectoderm. $\times$ 169.

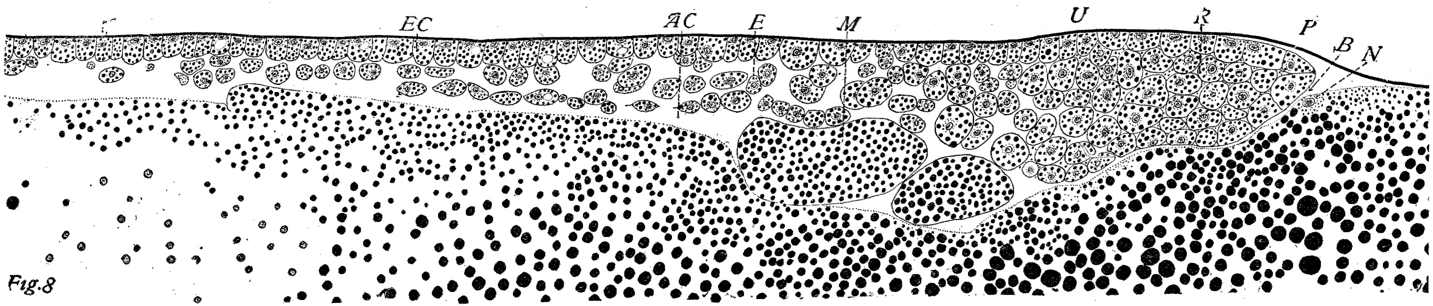

FIG. 8. Posterior portion of a median section taken through CD of Fig. 6. B, blastopore; R, dorsal lip of the blastopore; U, union between the deeper cells of the dorsal lip and the entoderm. $\times$ 169.

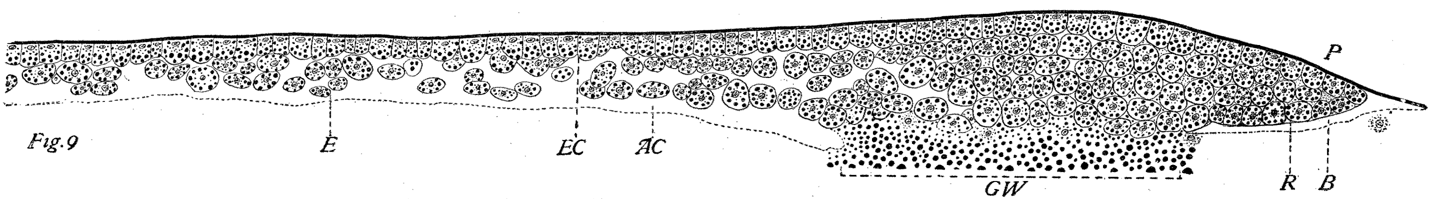

FIG. 9. Posterior portion of a section taken through EF of Fig. 6. It shows the lateral portion of the lip (R) and of the blastopore (B). $\times$ 169.

blastopore no periblastic nuclei are present, except a few at the extreme margin of the blastoderm (*N*).

Fig. 9 represents the posterior portion of a section taken through *EF* of Fig. 6. At the extreme right is the dorsal lip of the blastopore (*R*). This lateral part of the lip is not so thick as in the median section (Fig. 8, *R*). Below the lip is the lateral portion of the blastopore. The section also passes through the end of the right horn of the germ-wall (*GW*). The remaining structures are very similar to the corresponding parts of Fig. 8 and need no further description.

From Fig. 6 it will be seen that a section taken through *GH* would no longer contain any portion of the blastopore, since in this region the outer edge of the germ-wall reaches to the margin of the blastoderm. This section is represented in Fig. 10, and its most important part, consisting of a mass of cells from which the entoderm arises anteriorly, is shown at *D*. Between this mass and the inner edge of the germ-wall there is a space in which only a few cells are present. In some sections, however, no such space exists, but the mass of cells is directly continuous with the germ-wall. In such cases it is easy to gain the impression that the mass is a part of the germ-wall, and thus to be led astray into concluding that the entoderm arises directly from the germ-wall. A close study, however, shows that the character of this mass, even in cases of its most intimate union with the germ-wall, is such as to make it easy to distinguish the one from the other. As previously stated, many of the cells in the region of the germ-wall are directly open to the underlying yolk, in which periblastic nuclei are present, but the cells of the mass are entirely separated from the yolk and are completely delimited by cell-walls. The significance of this mass will be considered in connection with Fig. 13.

Fig. 11 is a median longitudinal section of a blastoderm taken thirty-eight hours after fertilization. It shows the condition of the blastoderm shortly after the closing of the blastopore, and clearly represents the character of the entoderm during the few hours immediately following this event. It will be seen that the entoderm is not a continuous layer, especially in the anterior region, where the cells are more or less in groups. Later, how-

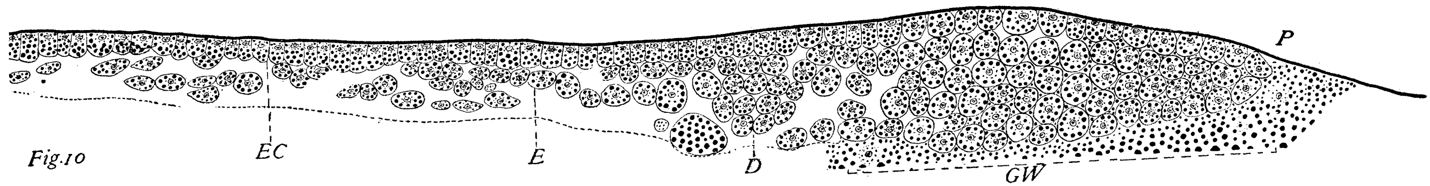

FIG. 10. Posterior portion of a section taken through GH of Fig. 6. D, mass of cells. $\times$ 169.

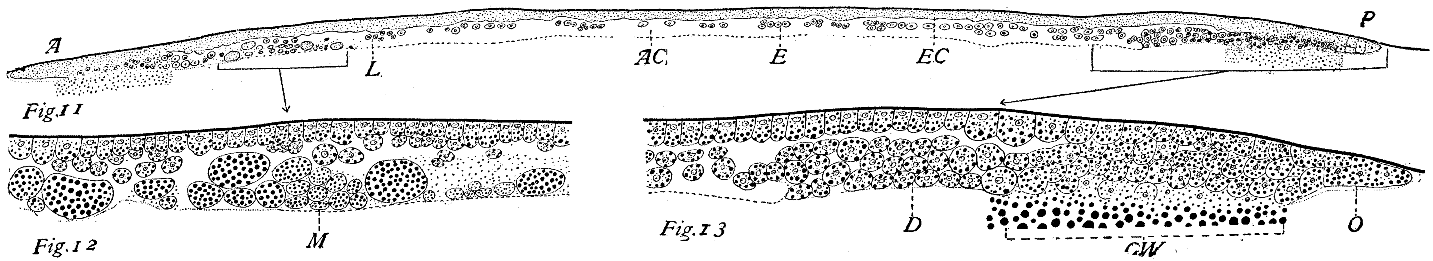

FIG. 11. A median longitudinal section of a blastoderm taken thirty-eight hours after fertilization, or three hours before laying. $\times$ 72.
FIG. 12. Enlarged anterior portion of the subgerminal cavity of the section represented in Fig. 11. $\times$ 169.
FIG. 13. Enlarged posterior portion of Fig. 11. $\times$ 169.

ever, these cells spread out and at the same time become flattened thus filling the intervening spaces and producing the characteristic gut-entoderm. At the stage represented in this figure, the entoderm in its forward growth has not yet reached the anterior limit of the subgerminal cavity, but its free edge ends about .35 mm. from this point (Fig. 11, *L*). The anterior part of the cavity not yet penetrated by the entoderm is occupied mainly by large yolk masses (Fig. 12). In the posterior part of this section the entoderm is directly continuous with the *mass* of cells (Fig. 13, *D*), which in turn is continuous with the inner edge of the germ-wall, and posterior to this wall is the region of overgrowth (*O*). In order to prove the origin and significance of this mass one must have recourse to experimental data. This form of evidence shows that the right and left halves of the dorsal lip of the blastopore grow toward each other and fuse in the median plane, that is, in the plane of the future longitudinal axis of the embryo. This movement of material from the lateral halves is not confined to the dorsal lip alone, but is participated in by the more lateral portions of the margin, that is, by the horns of the germ-wall. In the large majority of blastoderms, however, the right and left horns of the germ-wall do not turn in along the median line and fuse, but their free ends, upon meeting simply coalesce and grow out over the yolk[1] (see Fig. 6). It should also be borne in mind that as the horns are moving toward the median line, they are at the time being carried centrifugally by the expansion of the blastoderm. In this way the fused halves of the blastoporic lip are enclosed just anterior to the inner edge of the germ-wall, and hence the mass of cells, referred to above, is derived from the deeper portions of this enclosed lip. This is most apparent immediately after the ends of the horns have met, when the ectoderm is not yet differentiated from the underlying mass.

The movement of the two lateral halves of the posterior margin toward the median line and their simultaneous fusion must be regarded as a form of "concrescence"—the right and left halves

[1] In the cases in which a "marginal notch" is present, and in the rare cases such as that described by Whitman ('83) the horns of the germ-wall must also turn in and fuse.

of the dorsal lip representing the "homotypical" halves of the future embryo.[1]

A reconstruction of the blastoderm represented in Figs. 11–13 is shown in Fig. 14. The over-growth (*O*) and both zones of the germ-wall (*Y* and *Z*) completely encircle the blastoderm. Just anterior to the inner edge of the posterior germ-wall is the mass of cells (*R*) which is thick in the center, but gradually thins out

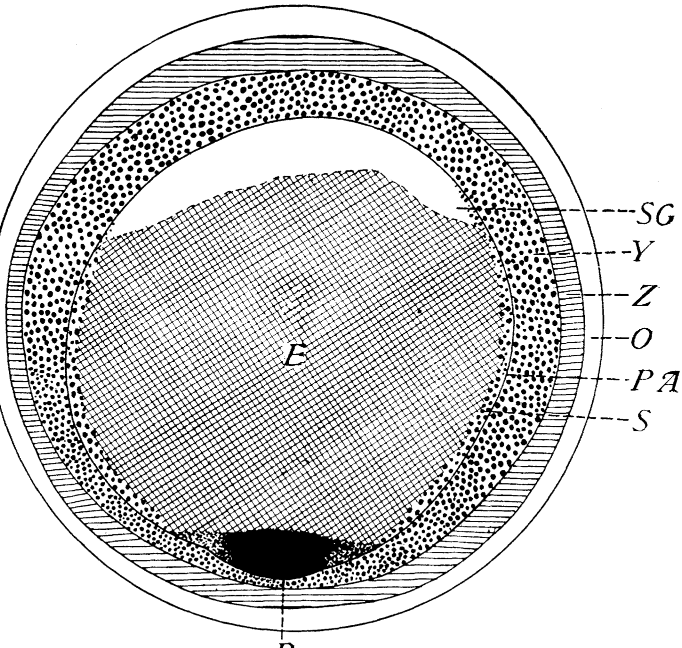

FIG. 14. A diagrammatic reconstruction of the blastoderm represented in Figs. 11–13. See Fig. 6 for the significance of the lettering. *R*, mass of cells, or the deeper cells of fused halves of the dorsal lip. × 27.2.

towards the sides.[2] Anteriorly it is continuous with the entoderm, into which it is differentiating as the latter grows forward through the subgerminal cavity. At this stage the entoderm (*E*) still ends with a free edge anteriorly, but its lateral margins are united with the yolk-sac entoderm at *S*. The entoderm does

[1] This view does not differ essentially from that advanced for the chick by Rauber ('76), Whitman ('78 and '83) and others — a view hitherto not supported by experimental workers, mainly because they experimented upon stages which were too far advanced.

[2] This mass probably corresponds to what Koller ('81) called a "Sichel."

not reach the anterior limit of the subgerminal cavity until from two to four hours after laying, at which time the mass disappears.

Origin of the Primitive Streak.

The primitive streak in the pigeon's blastoderm becomes visible in surface views between the fourth and fifth hours of incubation. In sections, however, it can be detected as early as two hours previous to this time. Its first appearance in section is that of small protuberances of cells on the under surface of the ectoderm situated along the median line. In their longitudinal extension these swellings reach from the posterior edge of the *area pellucida* to a point lying about half way between this edge and the center of the blastoderm (Fig. 15, *ps*). Under high magnification (Fig. 17, *ps*) these swellings are seen to be groups of rapidly dividing cells, which at first are separated from the gut-entoderm, but upon further growth come in contact with it. At the stage represented in Fig. 17 the gut-entoderm is a single layer of flattened cells and is directly continuous with the yolk-sac entoderm (Figs. 15–17, *Y*). The latter is thicker just posterior to the primitive streak than in any other region of the yolk zone.

The evidence afforded by a study of gastrulation indicates the line along which one must look for an explanation of the origin of the primitive streak. During the progress of concrescence there are laid down along the sides of the future longitudinal axis of the embryo strips of primary ectoderm, which are fused along the median line. These strips were previously the superficial cells of the right and left halves of the dorsal lip of the blastopore. For four or five hours after the closing of the blastopore, this median strip cannot be distinguished from the adjoining ectoderm. It is not until the protuberances begin to make their appearance that any difference can be seen, and even then, the double structure of the median region is not evident. In fact, it is only when the primitive groove appears that this bilateral structure becomes clear.

In conclusion, I may say, that during the process of gastrulation only the gut-entoderm is involuted; the chorda and mesoderm arise from the primitive streak, which represents the fused

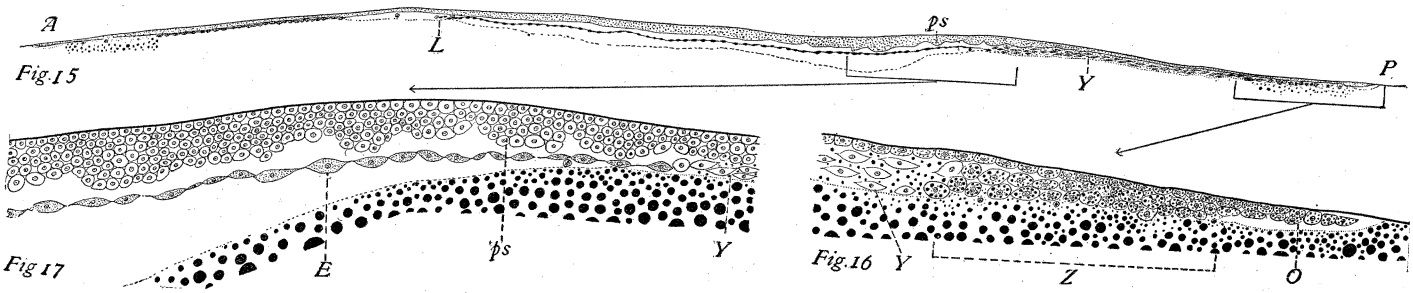

FIG. 15. A median longitudinal section of a blastoderm incubated four hours. It shows the appearance of the primitive streak as first seen in section. $\times 41$.

FIG. 16. Enlarged portion of the posterior end of Fig. 15. $\times 169$.

FIG. 17. Enlarged portion of the primitive streak region of Fig. 15. $\times 169$.

halves of the dorsal lip of the blastopore. The main evidence in support of this view is derived from experimental data, which will be presented in a future paper.

It gives me pleasure here to acknowledge my indebtedness to Professor Whitman, under whose direction the work has been carried on, and also to Professor F. R. Lillie for help and criticism.

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COMMON REFERENCE LETTERS USED IN THE FIGURES.

A Anterior end of the blastoderm.
AC Archenteric cavity.
B Blastopore.
E Invaginated or gut-entoderm.
EC Ectoderm.
F Floor of the subgerminal cavity.
GW Germ-wall.
L Anterior limit of the gut-entoderm.
M Yolk masses.
N Periblastic nuclei.
O Region of overgrowth.
P Posterior end of the blastoderm.
R Dorsal lip of the blastopore.
SC Segmentation cavity.
SG Subgerminal cavity.
V Vitelline membrane.
Y Yolk zone.
Z Zone of junction.

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